calcium and the receptor. The fraction of receptors in the ternary form is given by p, where

$$p = \frac{AM\,K_2K_3}{1+AK_1+MK_2+AM\,K_2K_3}$$

and where A and M are the concentrations of amiloride and calcium respectively, and where K_1 and K_2 are the affinity constants for amiloride and calcium with the receptor and K_3 is the affinity constant for amiloride and the receptor-metal complex. Other ions (Ln^{3+}, Mn^{2+}, Mg^{2+}, Sr^{2+}) are able to substitute for calcium.

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REFERENCES

CRABBÉ, J. & DE WEER, P. (1969). Relevance of sodium transport pool measurements in toad bladder tissue for the elucidation of the mechanism whereby hormones stimulate active sodium transport. *Pflüg. Arch. ges. Physiol.*, **313** 197–221.

GATZY, J. T. (1971). The effect of K^+-sparing diuretics on ion transport across the excised toad bladder. *J. Pharmac.*, **176**, 580–594.

Distribution of bound ^{3}H-benzilylcholine mustard in subcellular fractions of smooth muscle from guinea-pig ileum

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Studies on the uptake of the irreversible muscarinic blocking agent benzilylcholine mustard (BCM) (Gill & Rang, 1966) indicated that it would be a useful label for the muscarinic receptor (Rang, 1967). Preliminary experiments on the isolation of receptor material labelled with tritiated BCM are described.

Longitudinal muscle strips from guinea-pig ileum were exposed to 2×10^{-9} M ^{3}H-BCM for 30 min in Krebs solution at 37°C and then washed for 30 minutes. Pharmacological experiments show that this treatment causes about 95% receptor blockade.

The strips were then homogenized in 0·3 M sucrose and fractionated by differential centrifugation to yield three precipitates containing debris (P1; 3×600 *g* for 20 min); mitochondria (P2; $2\times10{,}000$ *g* for 20 min) and microsomes (P3; 100,000 *g* for 1 h) and a high-speed supernatant (SN3). Initially almost half of the protein and radioactivity was found in P1; however, all but about 8% of the activity could be removed from P1 by resuspending it and sonicating the suspension for 12 seconds. The microsomal fraction (P3) contained about 47% of the radioactivity and 11% of the protein. The distribution of acetylcholinesterase (Ellman, Courtney, Andres & Featherstone, 1961) and the membrane marker 5′-nucleotidase (Ipata, 1967; Song & Bodansky, 1967) closely paralleled that of the radioactivity in all fractions, suggesting that the radioactive label was attached mainly to cell membranes.

If 30 nM atropine is present during labelling very few receptors should be labelled by BCM, any uptake being due mainly to non-specific sites. Under these conditions it was found that uptake was reduced by 70%. The residual activity was distributed in the same way as the specific label which suggests that the non-specific sites were also on the membrane.

The P3 pellet was dissolved in 1% sodium dodecyl sulphate (SDS). Electrophoresis at pH7 on 5% polyacrylamide gels containing 1% SDS (Maizel, 1969) revealed three

radioactive peaks. The fastest-moving component had an apparent molecular weight of about 23,000 while a larger, less mobile peak had a M.W. in the region of 50,000 and could be a dimer of the smaller component. The third peak was found at the origin and varied considerably in size. It seems likely that this peak was due to aggregated material since it could be reduced considerably by prior treatment of the sample with the reducing agent dithiothreitol.

The results suggest that BCM is selectively bound to one or more protein components of smooth muscle cell membranes. The inhibition of this binding by low concentrations of atropine indicates that it may be related to muscarinic receptors.

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REFERENCES

ELLMAN, G. L., COURTNEY, K. D., ANDRES, V. & FEATHERSTONE, R. M. (1961). A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochem. Pharmac.*, **7**, 88–95.

GILL, E. W. & RANG, H. P. (1966). An alkylating derivative of benzilylcholine with specific and long-lasting parasympatholytic activity. *Mol. Pharmac.*, **2**, 284–297.

IPATA, P. L. (1967). A coupled optical enzyme assay for 5′-nucleotidase. *Analyt. Biochem.*, **20**, 30–36.

MAIZEL, J. V. (1969). Acrylamide gel electrophoresis of proteins and nucleic acids. In: *Fundamental Techniques in Virology*, ed. Habel, K. & Salzman, N. P. pp. 334–362. New York: Academic Press.

RANG, H. P. (1967). The uptake of atropine and related compounds by smooth muscle. *Ann. N.Y. Acad. Sci.*, **144**, 756–767.

SONG, C. S. & BODANSKY, O. (1967). Subcellular localization and properties of 5′-nucleotidase in the rat liver. *J. biol. Chem.*, **242**, 694–699.

Specific inhibitors for angiotensin II and angiotensin I

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Substitution of an unnatural amino-acid (Acpc: 1-Aminocyclo-pentanecarboxylic acid) for each of the eight amino-acids of the angiotensin molecule shows that 6-His and 8-Phe are essential for the activities of angiotensin II (AII) and angiotensin I (AI) on the arterial pressure (nephrectomized rats anaesthetized with urethane 1·4 g/kg s.c.) and on the rat isolated colon (Regoli & Vane, 1964).

Moreover, 8-Acpc AII, but not 6-Acpc AII antagonized the activities of AII and of AI on the two preparations.

Various analogues of angiotensin II substituted in position 8 have been tested for their inhibitory activities against AII and AI *in vivo* (rat blood pressure) and *in vitro* (rat isolated colon) (Table I).

TABLE 1. *Effect of 8 substituted angiotensin analogues on the pressor and myotropic (rat colon) action of* AII *and* AI

		Rat arterial pressure		Rat isolated colon	
Antagonist (Ant.)	Agonist (A)	Ratio Ant./A†	Inhibition %	Ratio Ant./A	Inhibition %
8 Acpc AII	AII	50	65±7	250	67±8
	AI	25	70±10	10	71±12
8 Ala AII	AII	50	60±5	125	82±7
(Park *et al.*, 1967)	AI	25	68±7	10	75±5
8 D-Phe AII	AII	100	52±8	500	50±11
	AI	50	50±11	20	48±9
8 Achc AII‡	AII	100	50±6	500–1000	70±13
	AI	50	53±5	20–40	65±11
4 Phe-8 Tyr AII	AII	50	58±10	5000	64±14
(Marshall *et al.*, 1970)	AI	25	40±7	100	20±7

Means of six experiments †Ratio for *in vivo* experiment has been calculated by dividing the dose of antagonist given by infusion ((μg/kg)min) by the dose of agonist given by injection (ng/kg). ‡Achc: A-Aminocyclohexanecarboxylic acid.